Summer 6-4-2012

Optimizing Pentose Utilization in Clostridia for Improved Solvents Production from Lignocellulosic Hydrolysates

Sheng Yang
Shanghai Research and Development Center of Industrial Biotechnology

Metabolic Engineering IX, Biarritz, France, 2012

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Shanghai Institutes for Biological Sciences

Chinese Academy of Sciences

Locations & historic changes of Chinese solvent plants

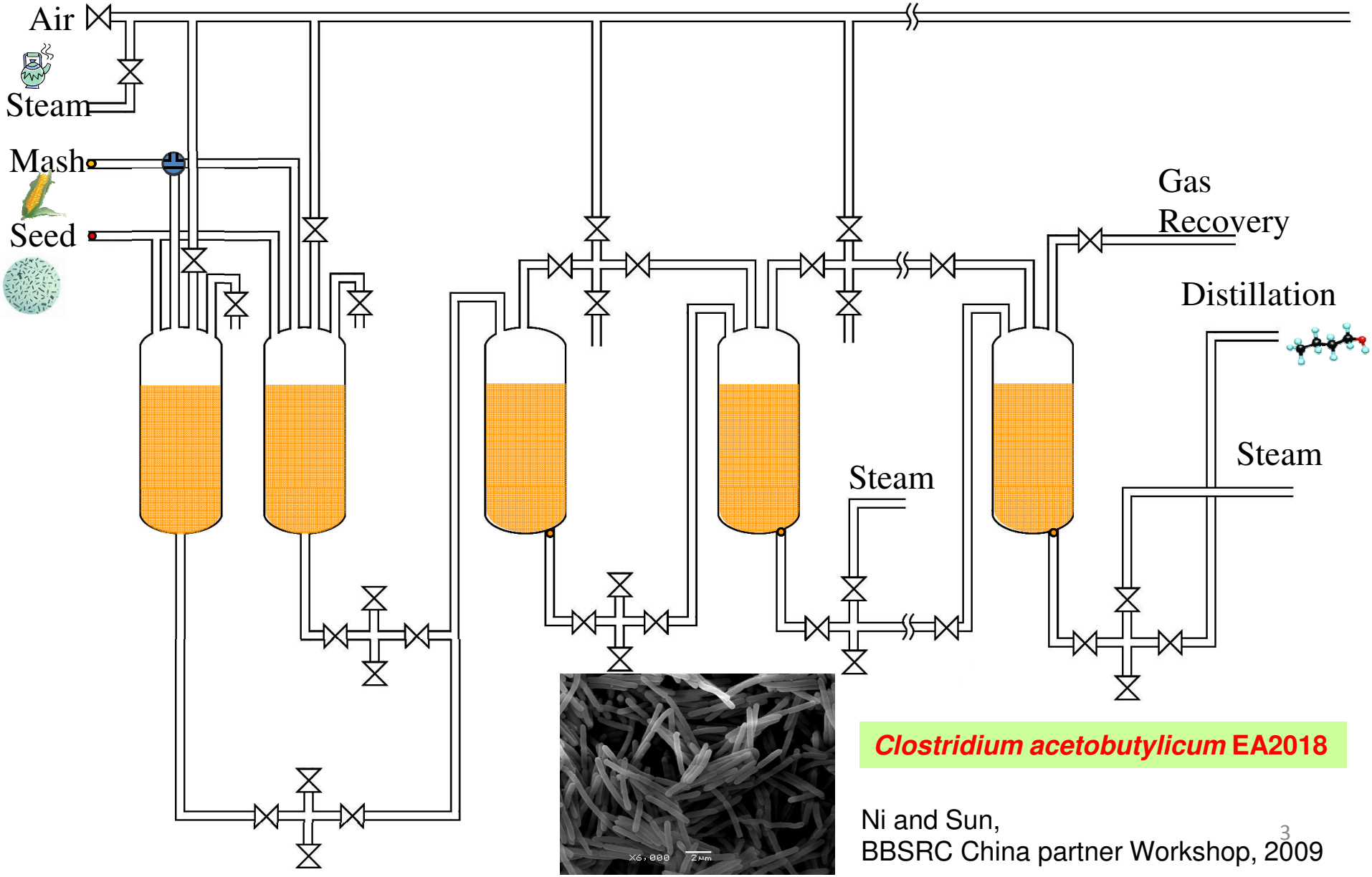
1st period plants, 1955-1965

2nd period plants, 1965-1985

3rd period plants, 1985-1996

Ni and Sun, BBSRC China partner Workshop, 200

Semi-continuous fermentation process for ABE production



***Clostridium acetobutylicum* EA2018**

Ni and Sun,
BBSRC China partner Workshop, 2009

Restoration of ABE fermentation in China from 2006

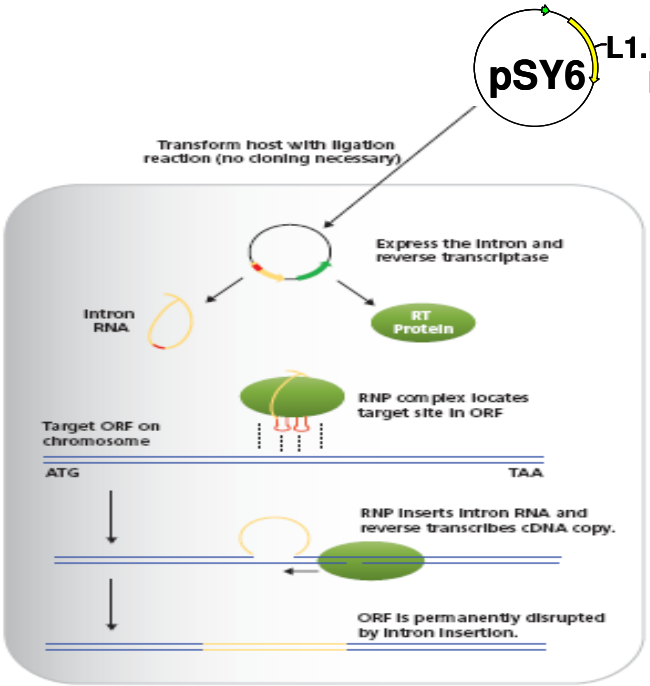
Laihe Chemical Co. Ltd

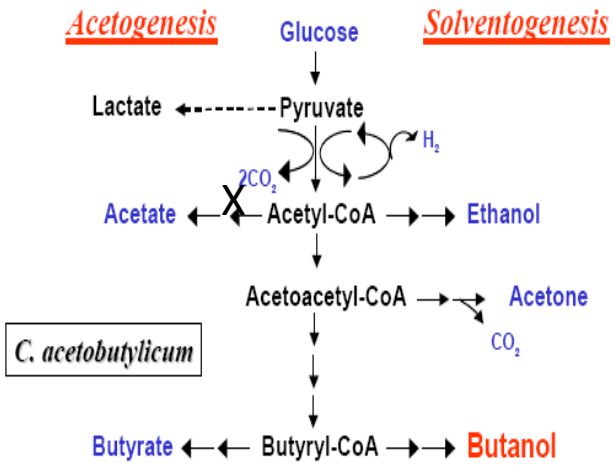
Companies	Ton/y	Location
Laihe Chemical Co. Ltd	100 000	Jilin
Jinyuan Ethanol Co. Ltd	50 000	Guangxi
Jiangsu Lianhai Biological Technology Co., Ltd.	50 000	Jiangsu
Lianyungang Lianhua	40 000	Jiangsu
Tianguan group	30 000	Henan
Cathay Industrial Biotech Ltd.	30 000	Jilin
The Jingmaoyuan Biochemical Company Ltd.	30 000	Jiangsu
Tongliao Zhongketianyuan Starch Chemical Corp.	10 000	Mengolia
Jilin Zhonghai Chemical	5 000	Jilin
Heilongjiang Haocheng Chemical	5 000	Heilongjiang
Jidong Solvent	5 000	Hebei
Hebei Jizhou Solvent	3 000	Hebei

Modified from Ni and Sun (2009)
Appl Micrbiol Biotechnol

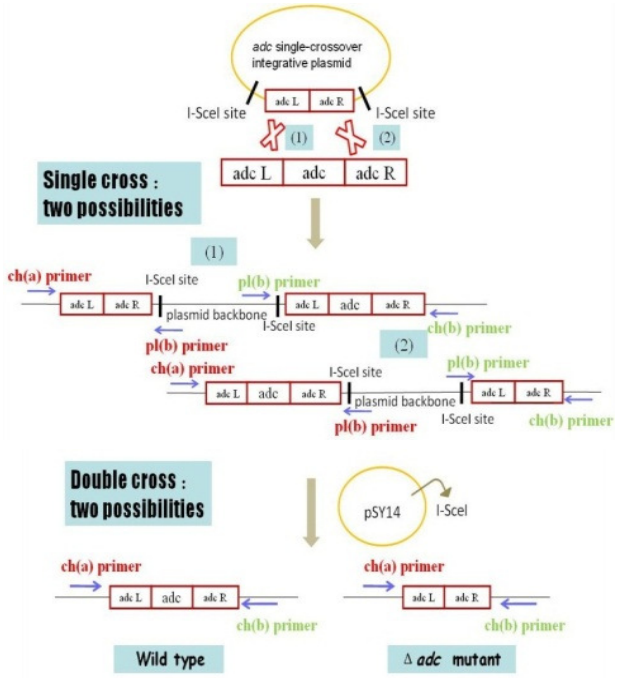
Gene disruption tools for Clostridia

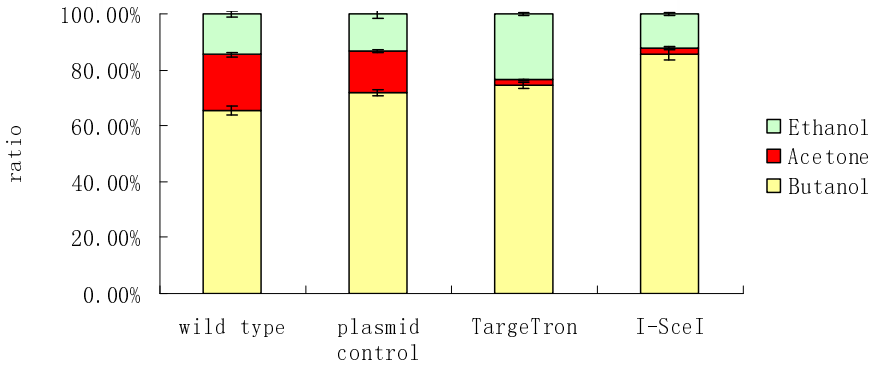
Group II Intron-based TargeTron





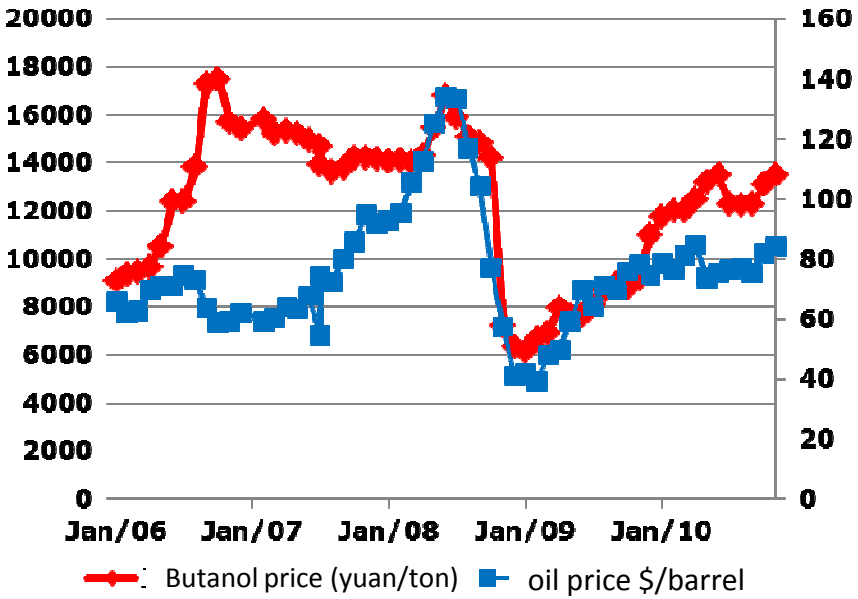
I-*Sce*I based markerless gene deletion

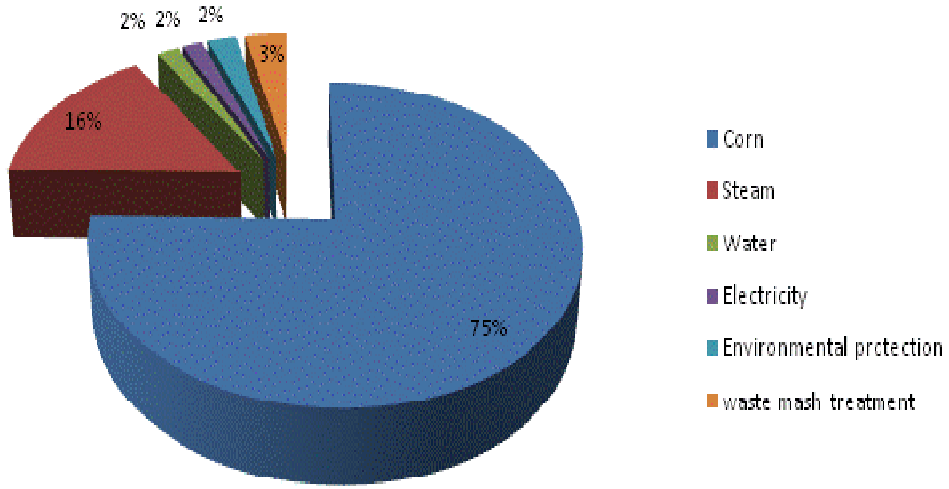




Heap J. et al (2007) J Microbial Method
Shao L. et al. (2007) Cell Research
Jiang Y. et al. (2009) Metabolic Engineering

Feedstock dominates the variable cost for solvent production



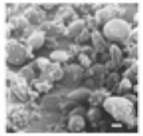


Distribution of variable cost for solvent production
data from NCPC

1.5 generation feedstock – non-grain hexose


Sweet sorghum


Cassava


Wheat amylodextrin


Molasses

Seeking lignocellulosic feedstock for ABE production

Sweet sorghum

Squeeze

Contionous Sterilization

Steam

Water

Enlarge cultivation

Fermentation

Distillation

300 t/a pilot

2.0 generation feedstock: pentose rich

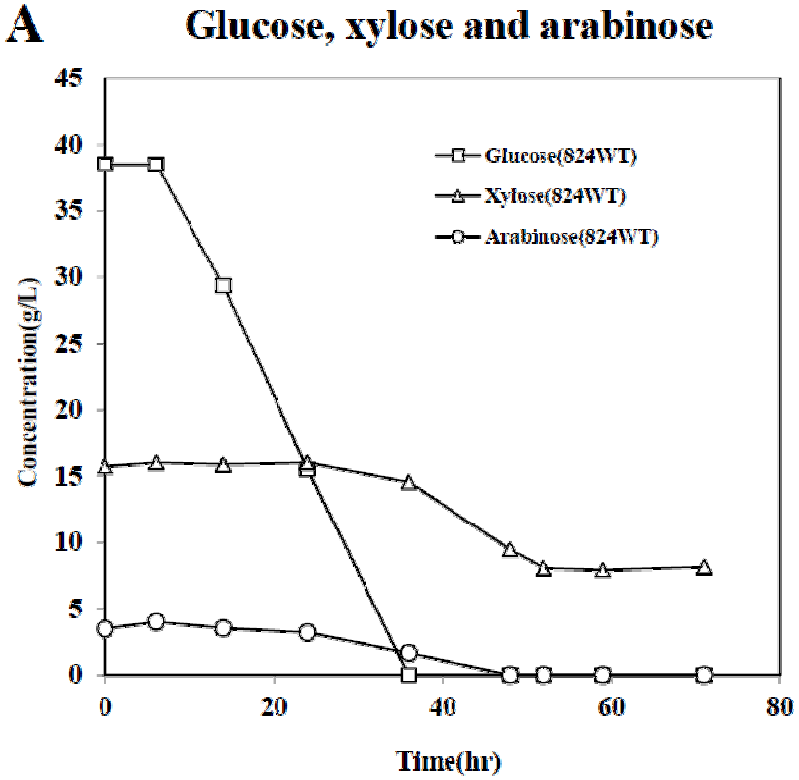
Sweet sorghum residue

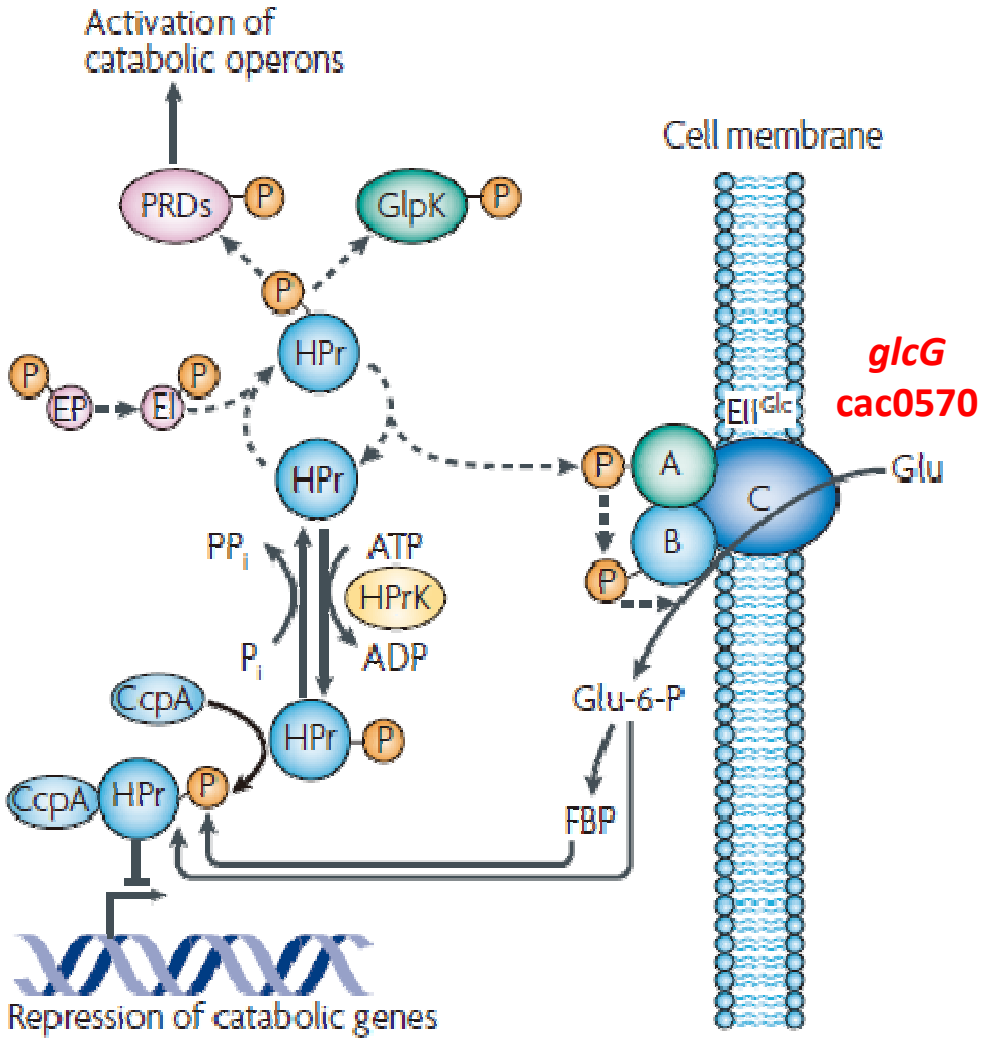
Corn stover

Corn cob

Corn fiber

Glucose repression in *Clostridium acetobutylicum*



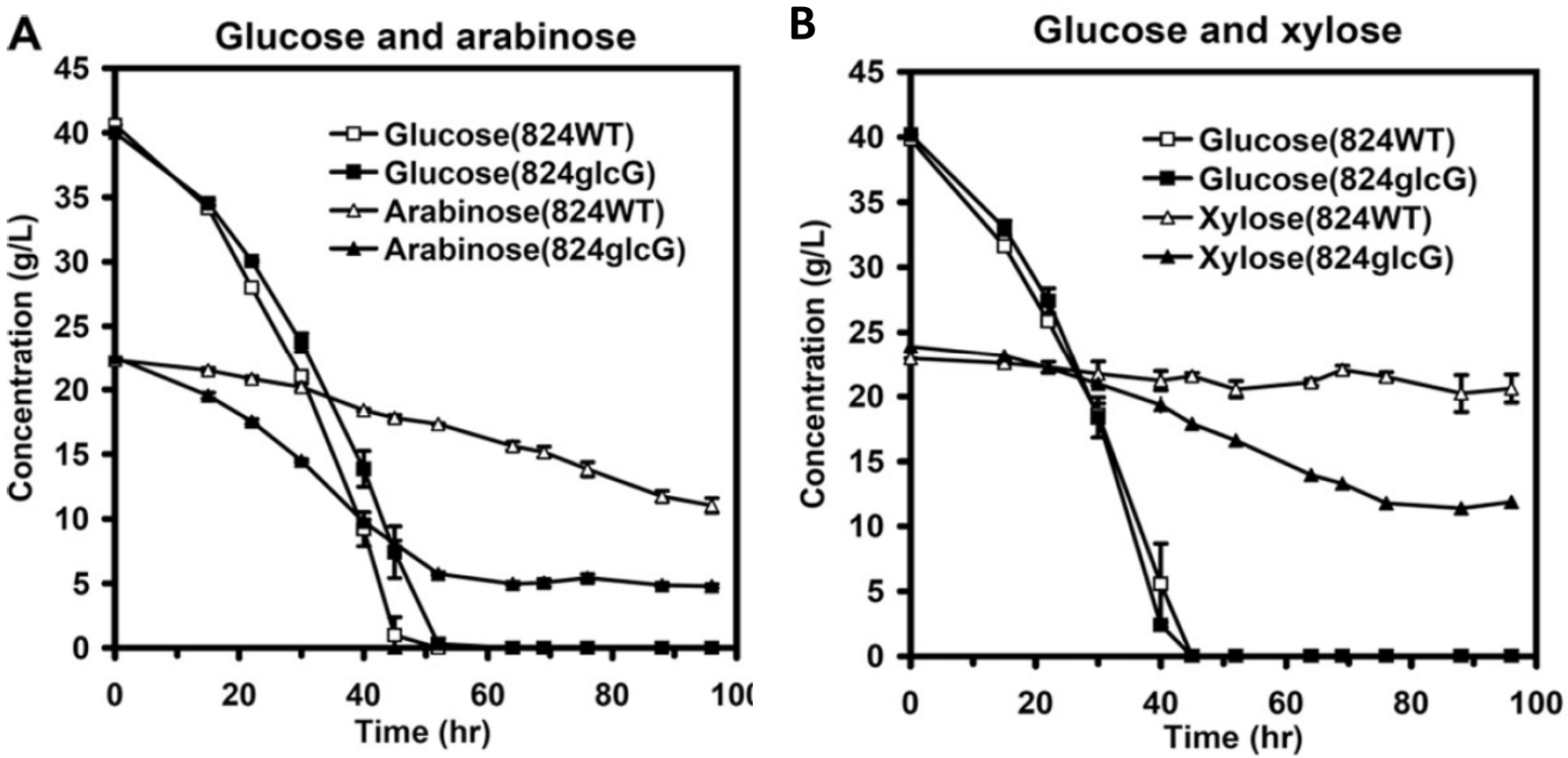


Carbon catabolite repression (CCR) in *Bacillus subtilis and other Firmicutes*

Gorke B (2008) Nat Rev Microbiol

Tangney M (2007) Appl Microbiol Biotech

Disruption of *glcG* relieve CCR effect in *C. acetobutylicum*



Rate-limiting steps of D-xylose metabolism occurs before PPP

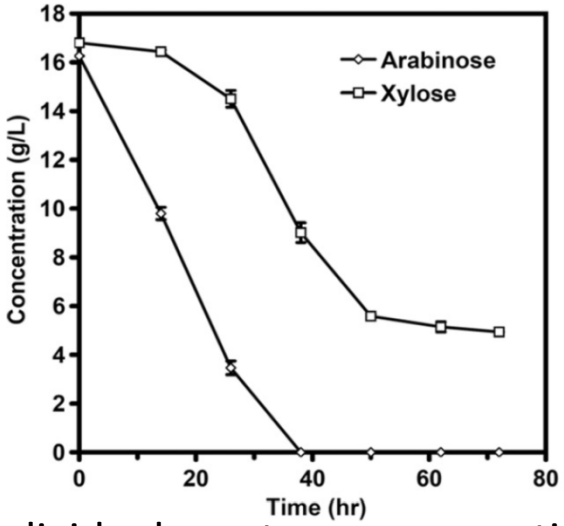


Fig. A Individual pentose consumption of 824WT

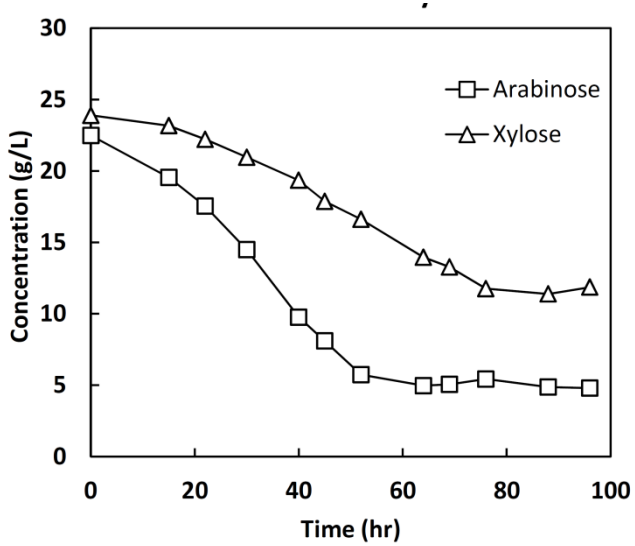
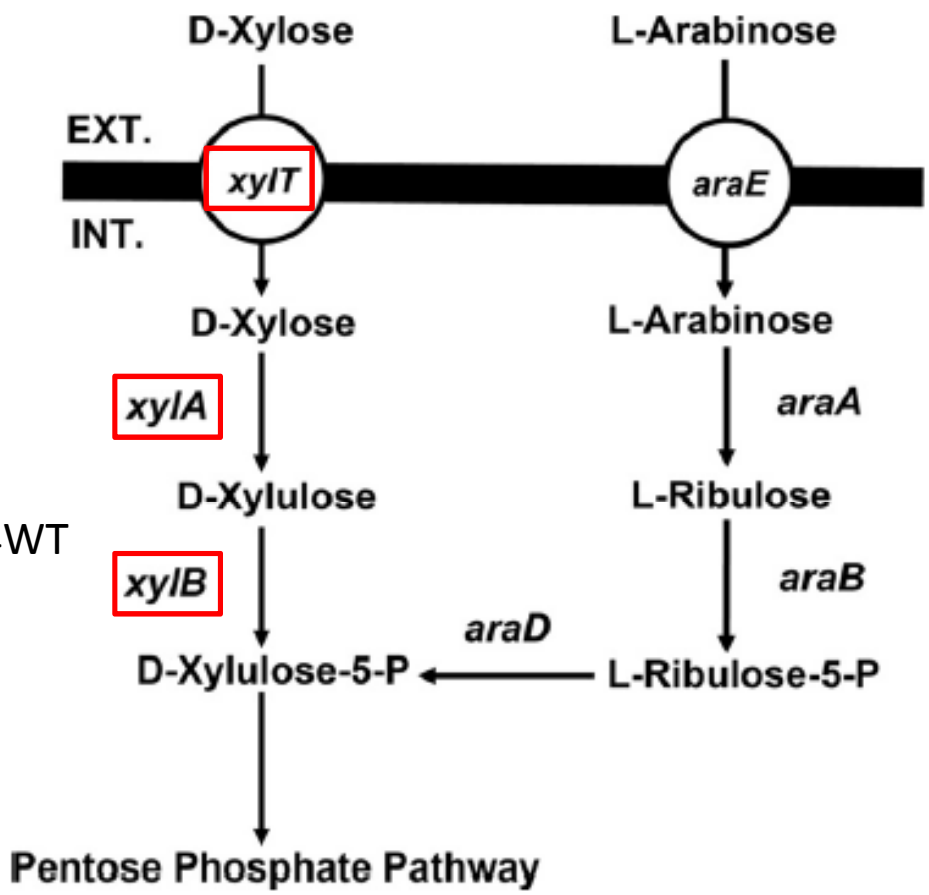
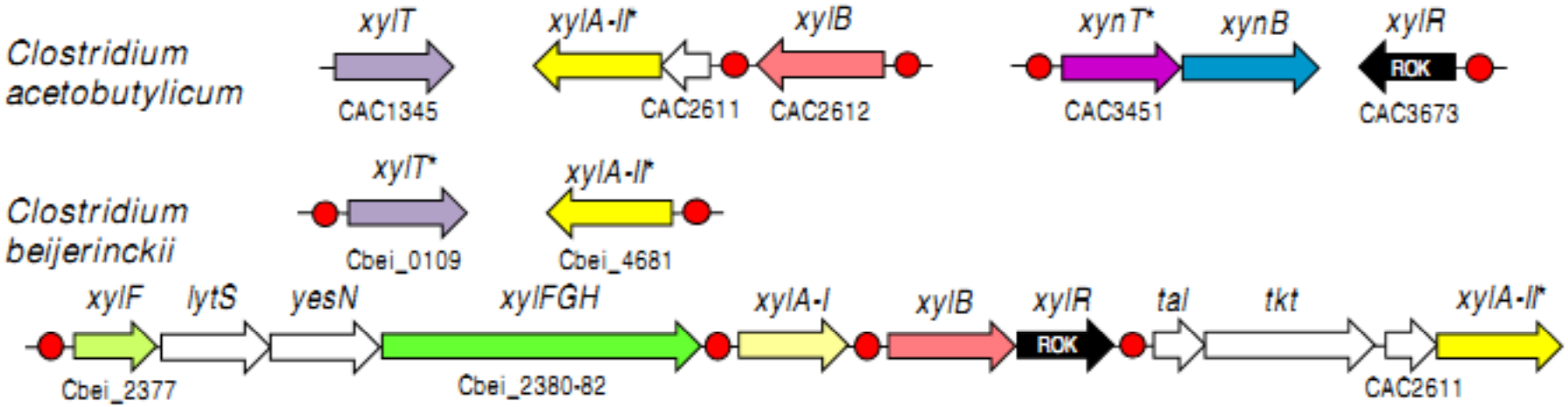


Fig. B Individual pentose consumption of 824glcG in presence of glucose



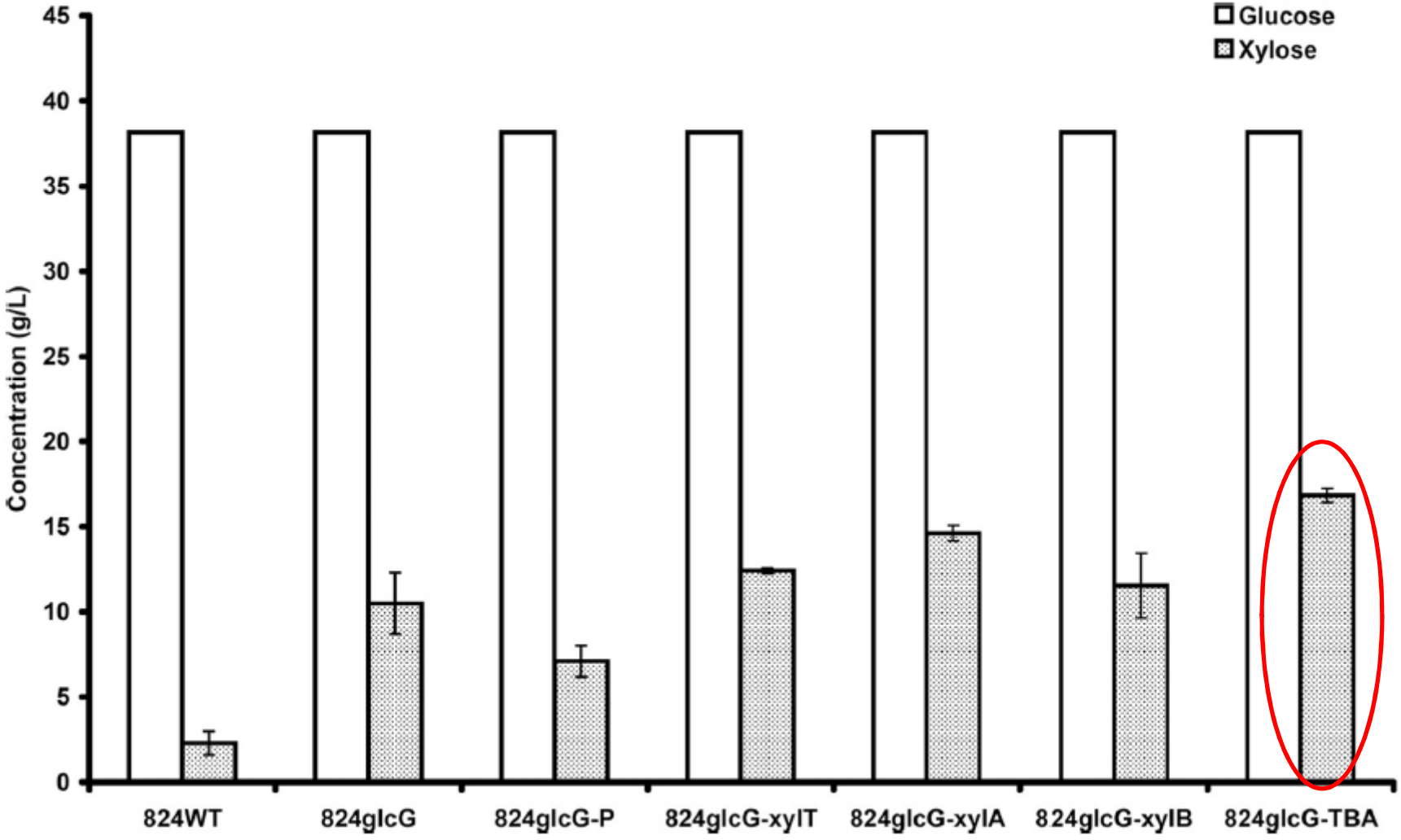
XlyR regulated genes in Clostridium genomes



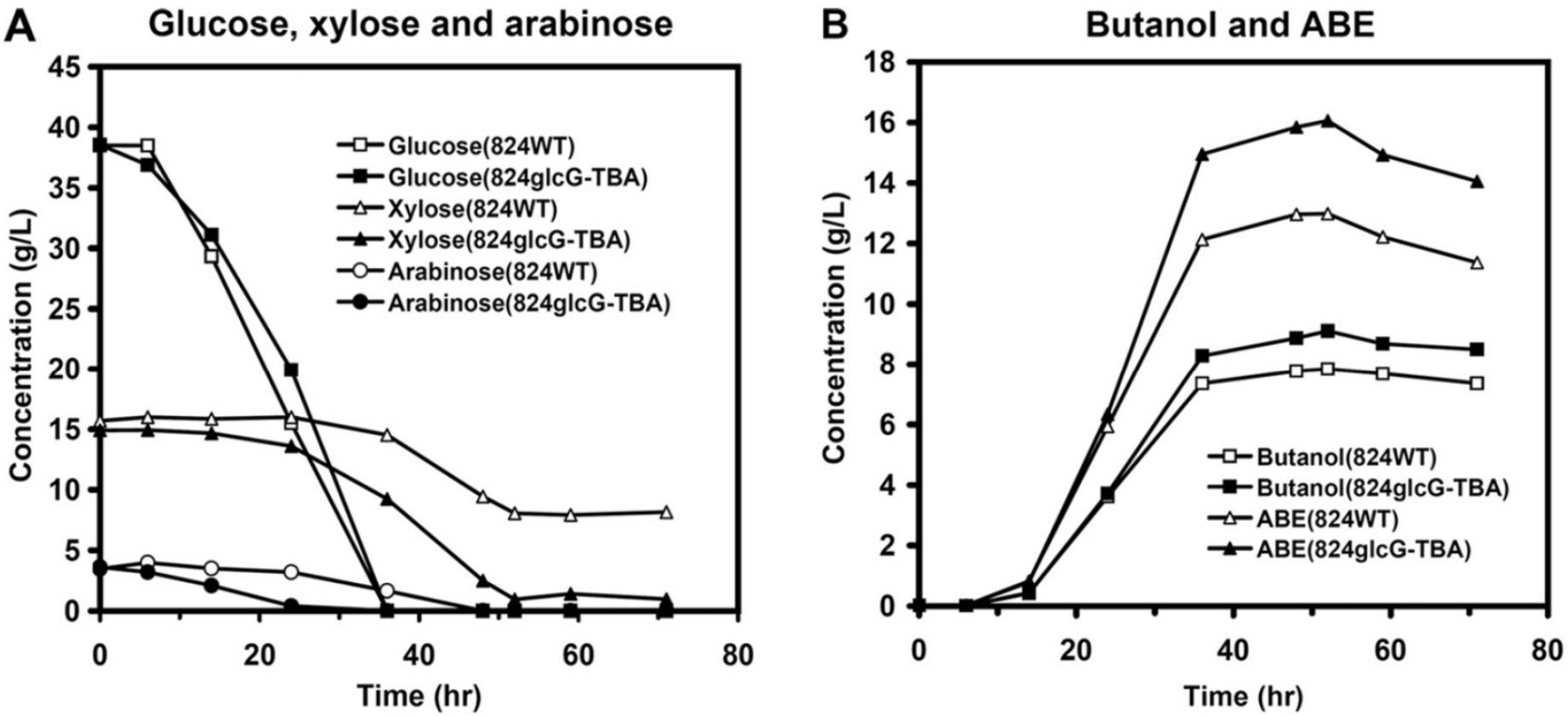
Candidate regulatory sites of XylR from ROK family are shown by red circles. Genes predicted by genome context analysis are marked by asterisks. Homologous genes are marked by matching colors.

Gu *et al* BMC Genomics 2010

Overexpression of *xylT, xylA* and *xylB* improved D-xylose consumption in the presence of D-glucose



Cofermentation of D-glucose, D-xylose and L-arabinose by 824glcG-TBA



Xiao et al 2011 AEM

Corncob-based xylose mother liquor is a pentose rich feedstock

山東龍力生物科技股份有限公司
SHANDONG LONGLIVE BIO-TECHNOLOGY CO., LTD.

Corncob

Acid hydrolysation

Neutralization

Ion-exchange

Concentration

60 g/L sugar

800 g/L sugar

liquid

Xylose mother liquor

500 g/L sugar

Solid

Xylose

wash

Xylose

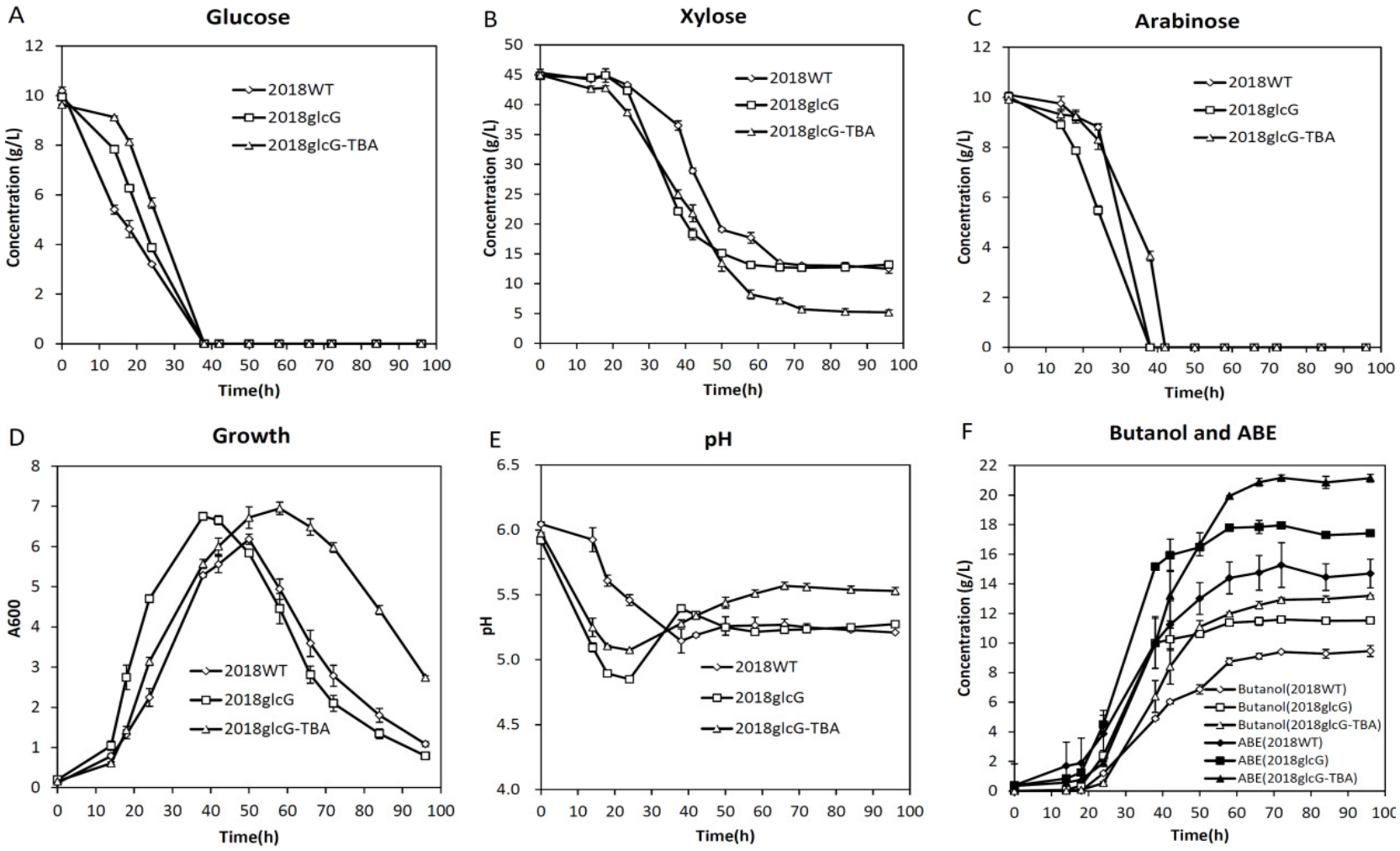
liquid

Xylose 70%
arabinose 15%
glucose 15%

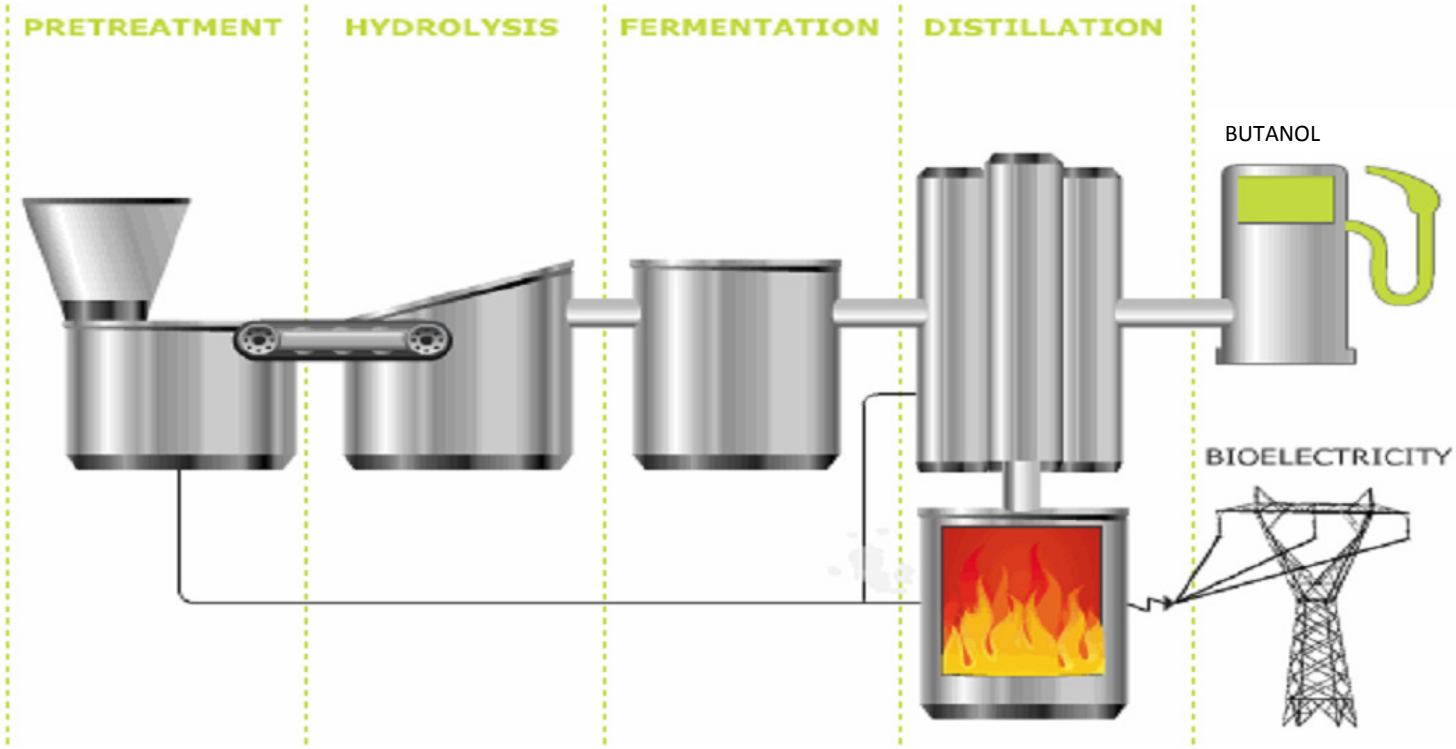
Fermentation profiles of strains 2018WT, 2018glcG and 2018glcG-TBA in xylose mother liquor

0.22 g/g

0.33 g/g



Can ABE be made from corn stover hydrolysates?



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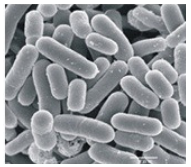


NCIMB8052 is promising starter strain in corn stover hydrolysates

Clostridia	growth	solvent (g/L)
C. acetobutylicum		
EA 2018	+	3.56
ATCC 824	+	3.14
C. beijerinckii		
NCIMB 8052	**+**	**11.2**
ATCC 55025	+	1.99
CGMCC 1.2127	-	
CICC 22954	-	
DSM 51	-	
DSM 791	-	
C. saccharobutylicum		
NCP 262	+	6.37
C. saccharoperbutylacetonicum		
N 1-4	-	

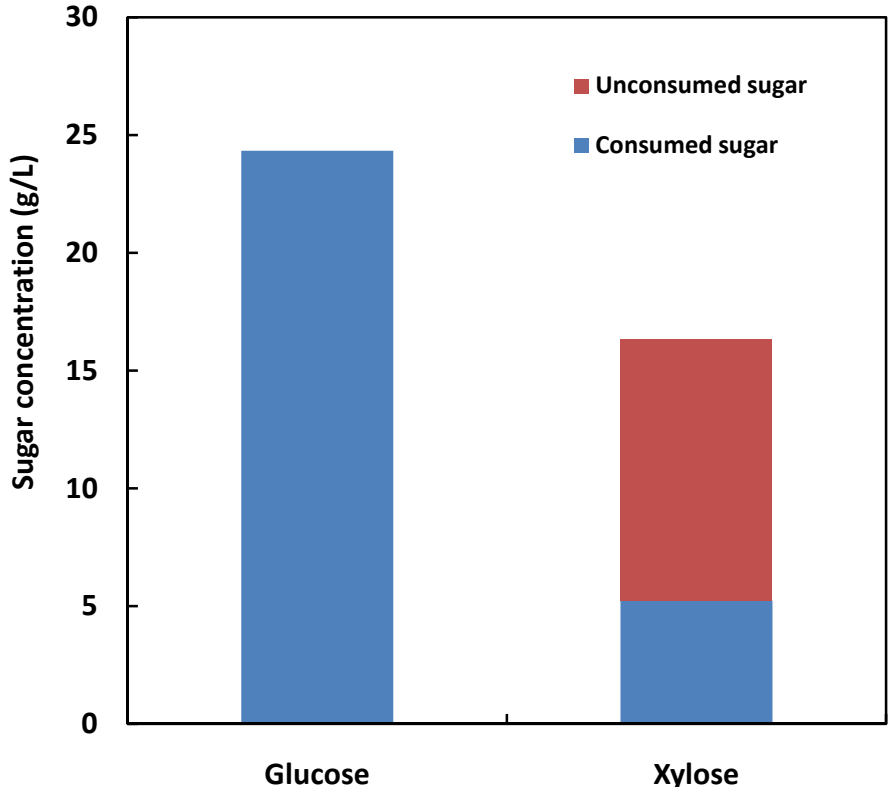
ABE fermentation of lignocellulose hydrolysates

Strain	Feedstock	Initial sugar (g/L)	Yield on total sugar (g/g)	Cost of medium (Yuan/per ton sugar)	Detoxification or not	Sugar utilization	Reference
C. beijerinckii ATCC55025	Wheat bran	45	0.21	1190	Y	Insufficient use of D-xylose and L-arabinose	(Liu, Ying et al. 2010)
C. Acetobutylicum ATCC824	Corn stover	45	0.27	1060	Y	Insufficient use of D-xylose and cellobiose	(Wang and Chen 2011)
C. beijerinckii P260	Barley straw	60	0.44	900	Y	Full use	(Qureshi, Saha et al. 2010)
C. beijerinckii P260	Corn stover	60	0.44	900	Y	Full use	(Qureshi, Saha et al. 2010)
C. beijerinckii IB4	Corn fiber	30	0.31	9800	N	Insufficient use of D-xylose and L-arabinose	(Guo, Tang et al. 2012)
C. beijerinckii P260	Wheat straw	25.4	0.37	2200	N	Full use	(Qureshi, Sahaa et al. 2008)
C. beijerinckii BA101	Corn fiber	25	~0.35	2130	N	Full use	(Qureshi, Ezeji et al. 2008)
C. beijerinckii P260	Wheat straw	28.4	0.42	2100	N	Full use	(Qureshi, Sahaa et al. 2008)
C. acetobutylicum P262	Corn stover	81	0.27	1300	N	74% of total sugars was consumed	(Parekh, Parekh et al. 1988)
C. beijerinckii P260	Wheat straw	52	0.35	1080	N	87% of total sugars was consumed, with D-xylose, and D-glucose left	(Qureshi, Sahaa et al. 2008)

The technical successful criteria

undetoxified, additive <200 yuan/t ABE, unsterilized, < 48 hour, titer>12g/L, yield>0.3 g/g total sugar

8052 only give a yield of 0.24 g/g in CSH

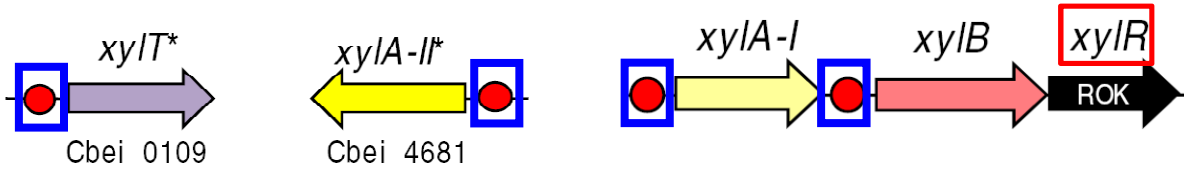


unwashed NREL PCS, CTec2 hydrolysates

Cellobiose (g/L)	Glucose (g/L)	Xylose (g/L)	acetic acid (g/L)	$SO4^{2-}$	Furfural (g/L)	HMF (g/L)
3.9	85.8	56.6	8.4	10.0	0.07	2.6

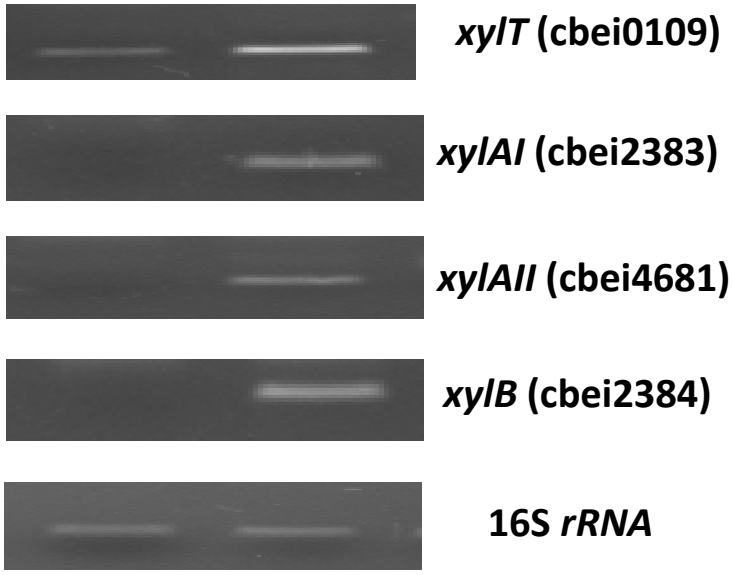
diluted to 40 g/L sugar, with 2 g/L ammonia sulfate, 5 g/L calcium carbonate
fermentation at 37°C for 48 hour

Disruption of gene *xylR* improves D-xylose utilization by *C. beijerinckii*



Candidate regulatory sites of XylR from ROK family are shown by red circles
Gu (2010) BMC Genomics

8052WT 8052ΔxylR



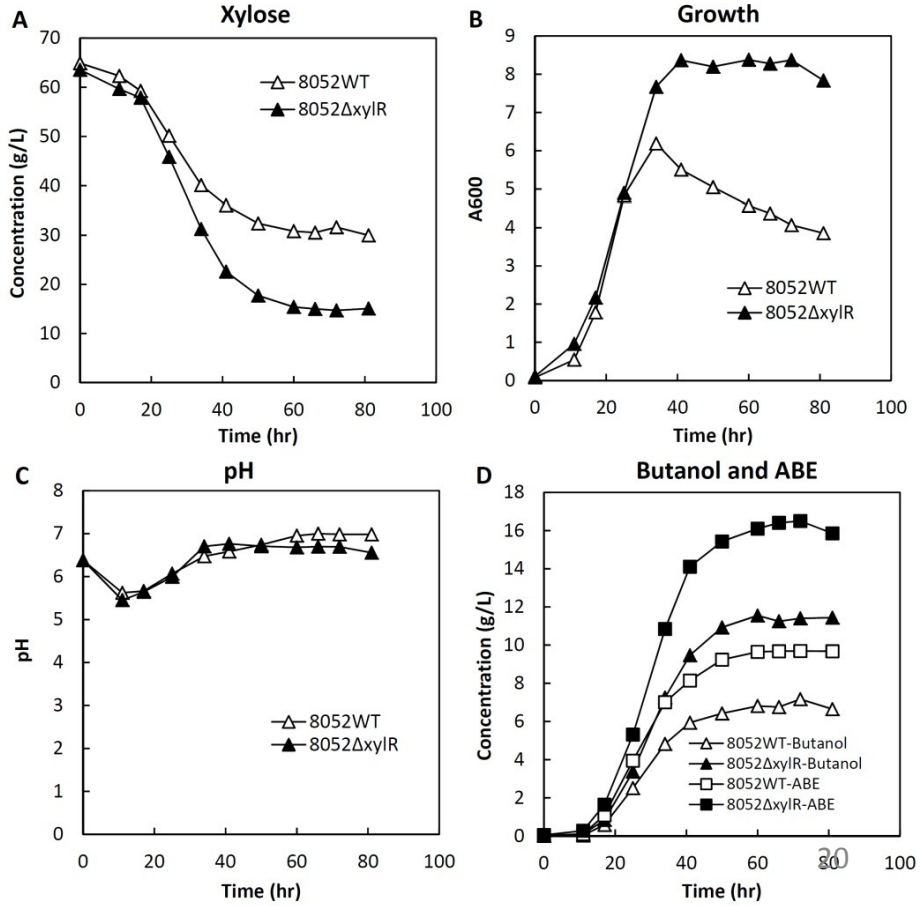
***xylT* (cbei0109)**

***xylAI* (cbei2383)**

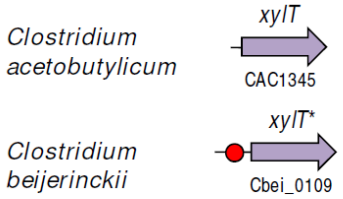
***xylAII* (cbei4681)**

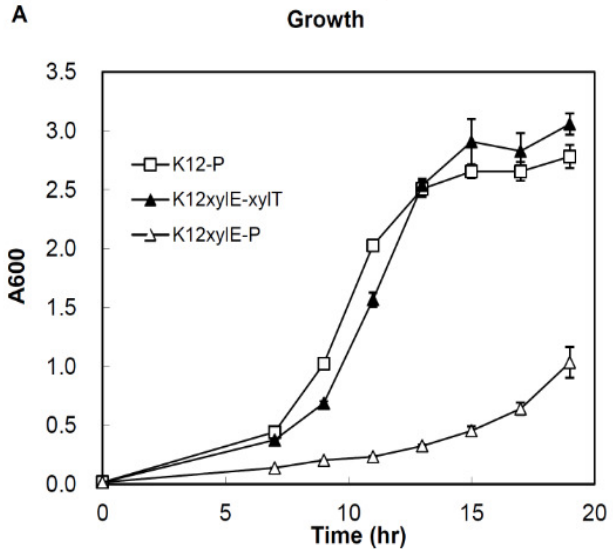
***xylB* (cbei2384)**

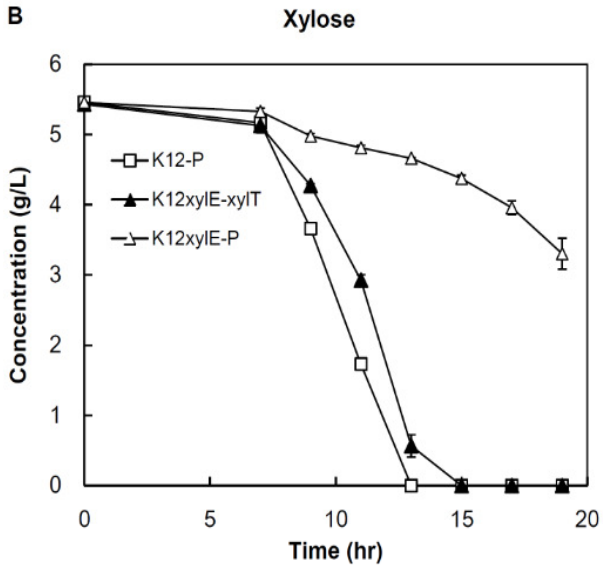
16S *rRNA*

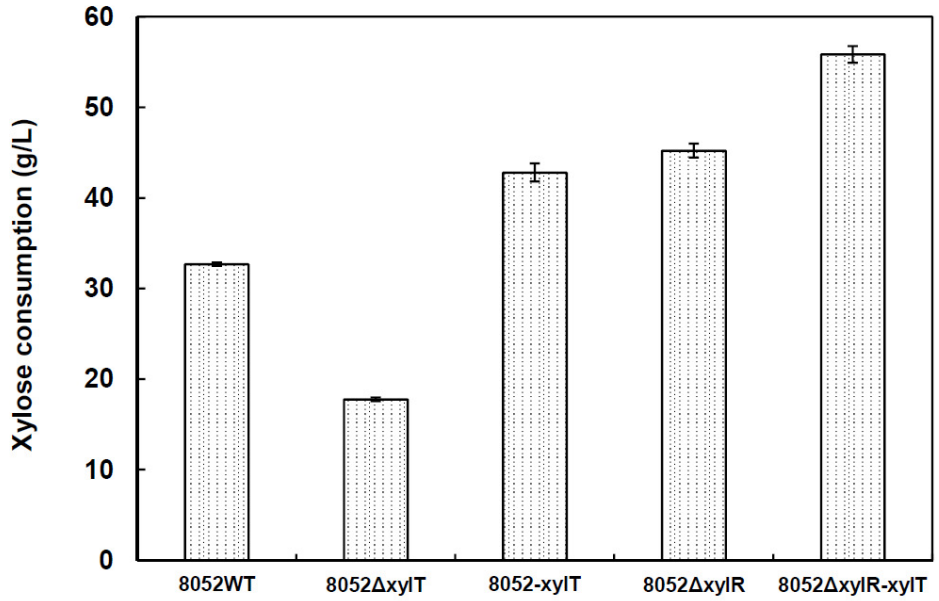


XylT overexpression in strain 8052ΔxylR further enhances D-xylose consumption





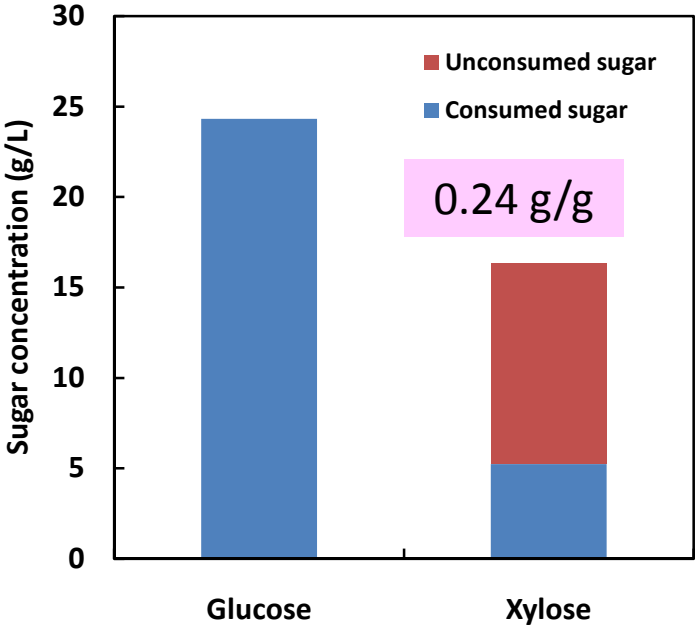




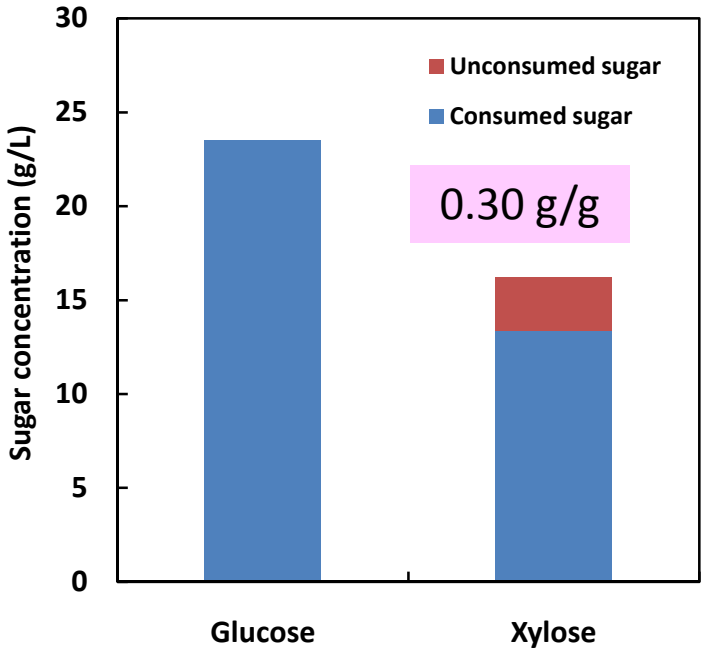
Xiao 2012 Metab Eng

8052ΔxylR-xylT (MM1643) produce 20% more solvent in CSH

8052wt in CSH



MM1643 in CSH



unwashed NREL PCS, CTec2 hydrolysates

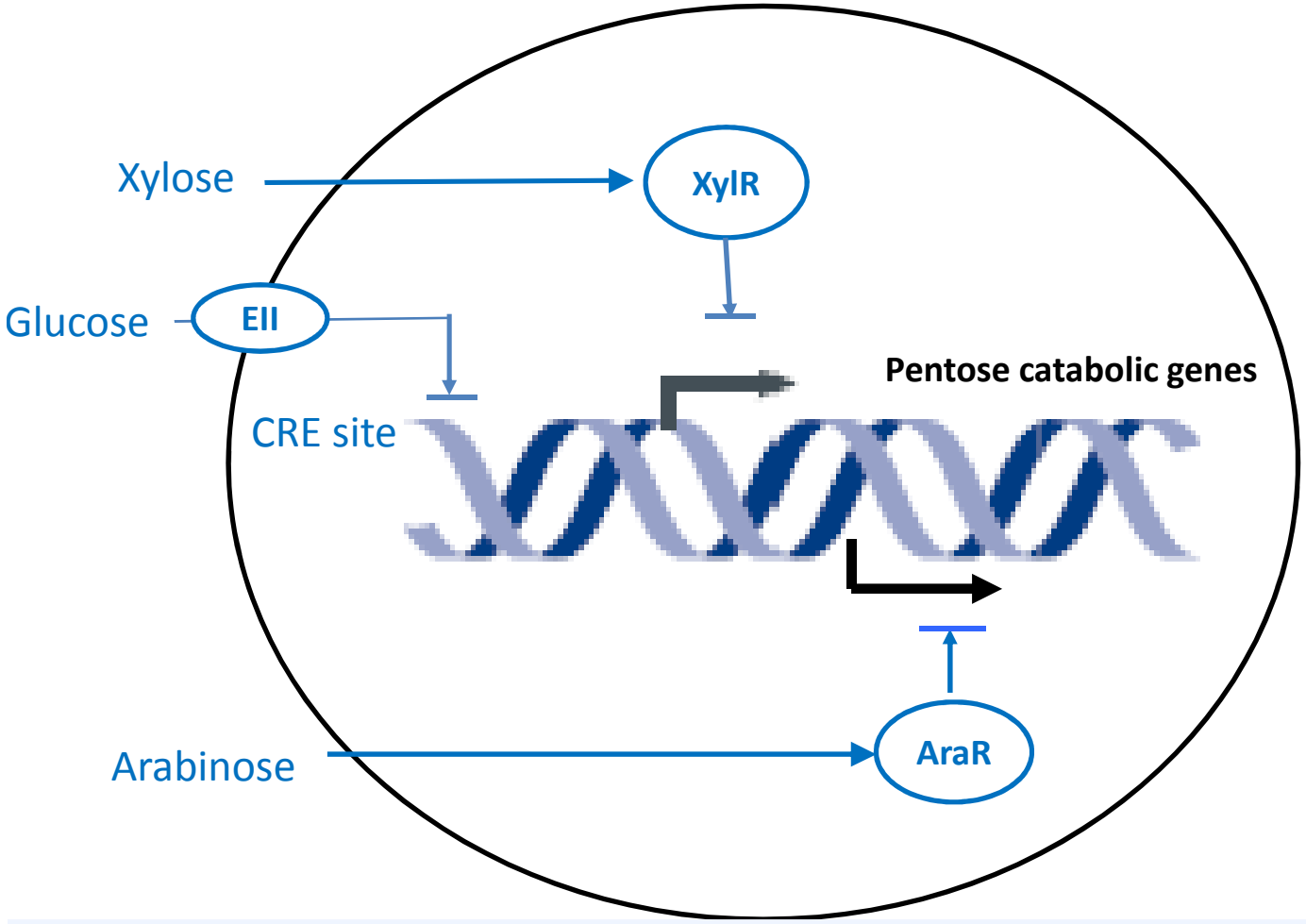
Cellobiose (g/L)	Glucose (g/L)	Xylose (g/L)	acetic acid (g/L)	$SO4^{2-}$	Furfural (g/L)	HMF (g/L)
3.9	85.8	56.6	8.4	10.0	0.07	2.6

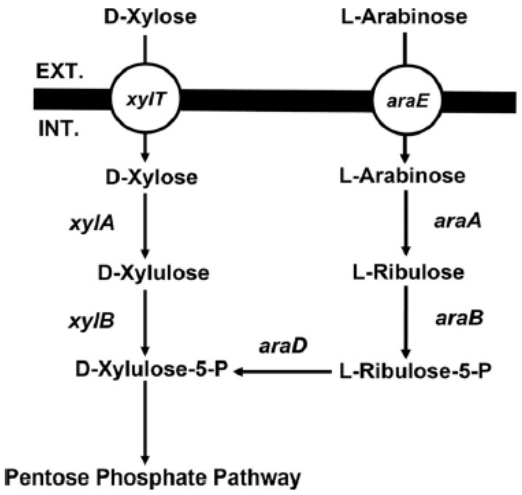
diluted to 40 g/L sugar, 2g/L ammonia sulfate, 5g/L calcium carbonate
fermentation at 37°C for 48 hour

Summary

- ABE fermentation in China is shifting from hexose to pentose-rich lignocellulosic feedstock.
- The genes of pentose metabolic pathway of two model strains, *C. acetobutylicum* ATCC 824 and *C. beijerinckii* NCIMB 8052, were identified and improved to enhance solvent yield by elimination the negative regulation as well as strengthen the xylose pathway.
- *Clostridium acetobutylicum* EA2018 can be engineered to coferment hexose and pentose of XML at solvent yield>0.3 by disruption of *glcG* and overexpression of xylose uptake and metabolism genes.
- CSH tolerant *Clostridium berjerinckii* NCIMB 8052 can be engineered to coferment hexose and pentose of undetoxified CSH at solvent yield>0.3 by disruption of *xylR* and overexpression of xylose transporter.

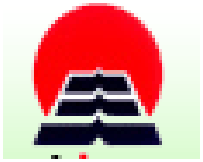
Strategy for Firmicutes pentose metabolic engineering





1. **Disrupt Enzyme II of PTS system** to release glucose repression
2. **Disrupt repressor protein** to enhance individual secondary substrate pathway
3. **Overexpression secondary substrate uptake and pathway genes**

Acknowledgement







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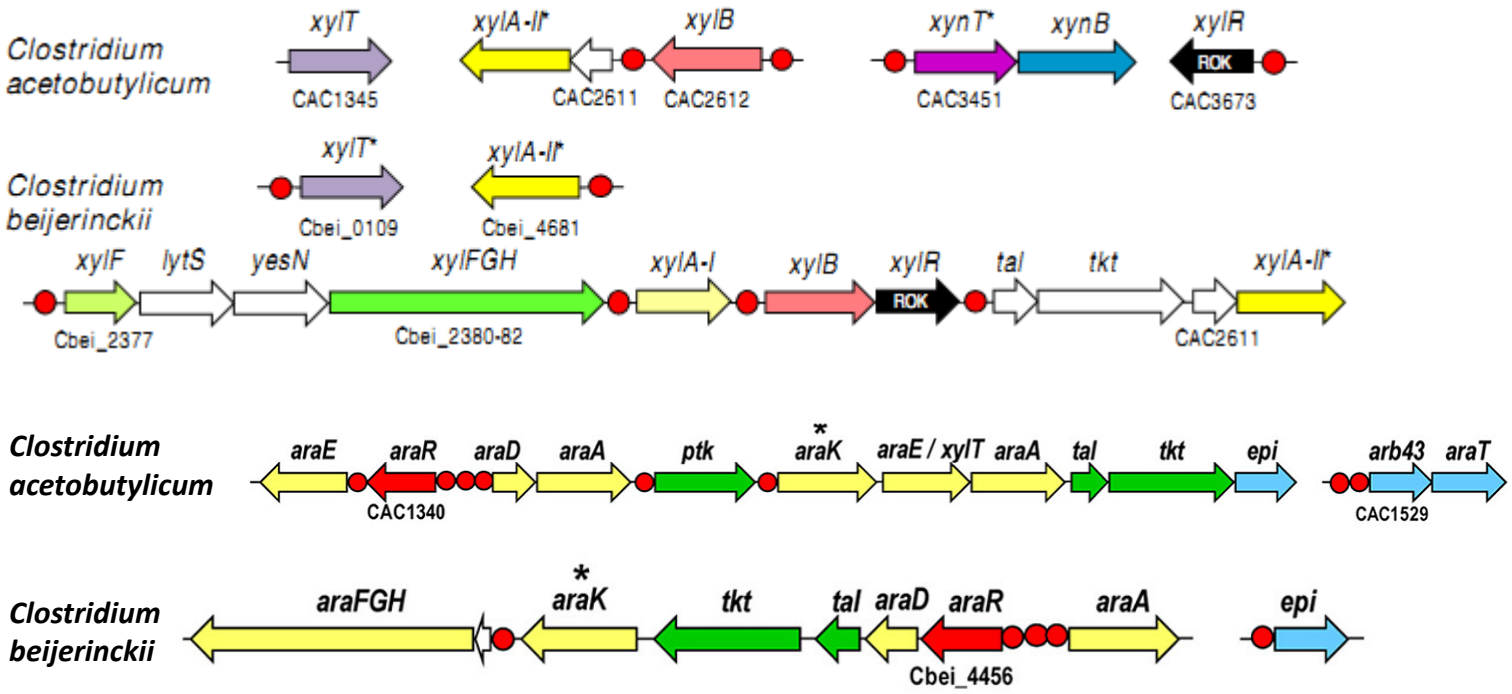
Prof Yunliu Yang
Prof Weihong Jiang
Prof Chen Yang

Prof ET Papoutsakis
Prof Peter Durre
Prof Nigel Minton
Dr Xinyan Guo
Dr Guifang Wu

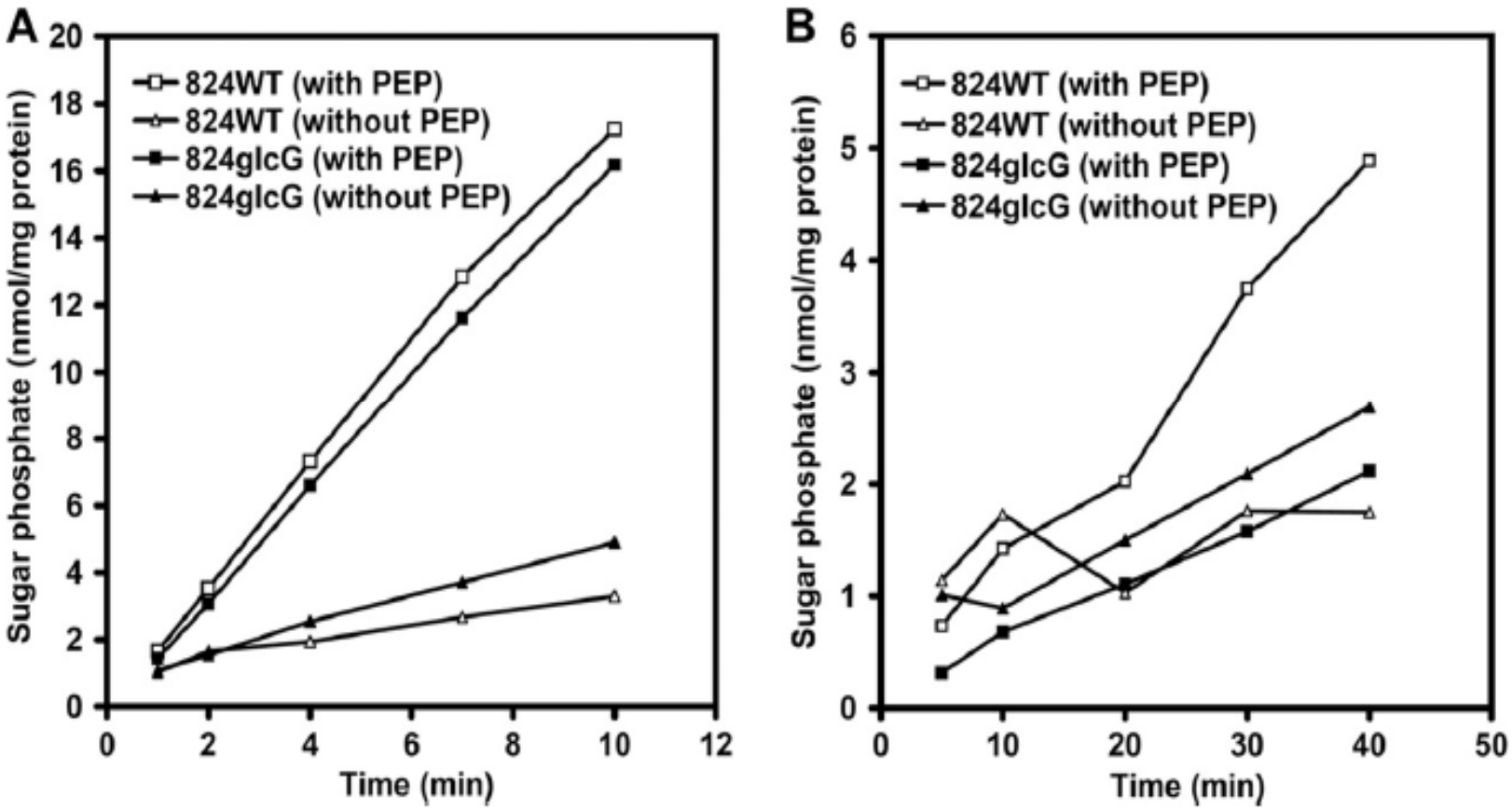
XlyR or AraR-regulated genes in Clostridium genomes



Candidate regulatory sites of XylR or araR from ROK family are shown by red circles. Genes predicted by genome context analysis are marked by asterisks. Homologous genes are marked by matching colors.

Gu *et al* BMC Genomics 2009
Zhang *et al* J Bacteriol 2012

I. The glucose-PTS activity was only slightly reduced in strain 824glcG and its methyl α-glucoside-PTS activity was lost



From Dr. Wilf

I. Non-PTS-mediated of D-glucose transport and metabolism might play a greater role in train 824glcG

TABLE 2. Comparison of specific activities of glucose kinase for strain 824WT and strain 824glcG in P2 medium containing 40 g/L glucose.

Strain	Specific activity (U/mg)[a]	
	Acidogenic phase	Solventogenic phase
824WT	0.16 ± 0.02	0.23 ± 0.06
824glcG	0.31 ± 0.13	1.05 ± 0.36

[a] OD_{600} of samples taken in acidogenic phase and solventogenic phase were around 1.8 and 4.0, respectively.

I. Transcriptional fold-changes of *xylT, xylA* and *xylB* of strain 824glcG-TBA

TABLE 3. Transcriptional fold-changes of *xylT*, *xylA* and *xylB* of strain 824glcG-TBA as compared to stain 824glcG-P.

Genes	Transcriptional level	
	Acidogenic phase[a]	Solventogenic phase[a]
xylT	1.17 ± 0.08	6.60 ± 0.56
xylA	132 ± 27	30 ± 3
xylB	119 ± 5	62 ± 4

[a] OD_{600} of samples taken in acidogenic and solventogenic phase were 3.8 and 7.0, respectively. Fermentations were performed in P2 medium containing 40 g/L glucose and 20 g/L xylose.